Southwestern Corn Borer (Lepidoptera: Crambidae) Damage and Aflatoxin Accumulation in Maize

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ABSTRACT Aflatoxin, a potent carcinogen, is produced by the fungus *Aspergillus flavus* Link: Fr. Drought, high temperatures, and insect damage contribute to increased levels of aflatoxin contamination in corn, *Zea mays* L. Plant resistance is widely considered a desirable method of reducing aflatoxin contamination. Germplasm lines with aflatoxin resistance have been developed. This investigation was undertaken to determine whether crosses among these lines exhibited resistance to southwestern corn borer, *Diatraea grandiosella* Dyar, and to assess the effects of southwestern corn borer feeding on aflatoxin accumulation. Differences in ear damage among southwestern corn borer infested hybrids were significant. Estimates of general combining ability effects indicated that the lines Mp80:04, Mp420, and Mp488 contributed to reduced ear damage, and SC213 and T165 contributed to greater damage when used in hybrids. Mean aflatoxin levels were 254 ng/g for hybrids infested with southwestern corn borer larvae and 164 ng/g for noninfested hybrids in 2000 when environmental conditions were conducive to aflatoxin production. In contrast, the overall mean aflatoxin level for southwestern corn borer infested hybrids was only 5 ng/g in 1999 when environmental conditions did not favor aflatoxin accumulation. Crosses that included lines selected for aflatoxin resistance as parents (Mp80:04 and Mp313E) exhibited lower levels of aflatoxin contamination both with and without southwestern corn borer infestation in 2000. Only the experimental line Mp80:04 contributed significantly to both reduced southwestern corn borer damage and reduced aflatoxin contamination.

KEY WORDS southwestern corn borer, aflatoxin, corn, plant resistance

AFLATOXIN, A NATURALLY occurring toxin produced by the fungus *Aspergillus flavus* Link: Fr., is a potent carcinogen (Castegnaro and McGregor 1998, Pittet 1998). The marketability of corn (*Zea mays* L.) grain is greatly reduced if contaminated with aflatoxin. The U.S. Food and Drug Administration has set a tolerance of 20 ng/g for aflatoxin B_1, the most common form of aflatoxin in corn. Grain with higher levels is restricted from interstate commerce (Gourma and Bullerman 1995).

Contamination of corn with aflatoxin is a frequent problem in the Southeast and a sporadic problem in the Midwest (Payne 1992, Widstrom 1996). Drought, high temperatures, and insect damage are often associated with high levels of aflatoxin contamination (Guthrie et al. 1981, McMillian et al. 1985, Diener 1989, Payne 1992, Dowd 1998).

Plant resistance is generally considered a highly desirable method of reducing aflatoxin contamination in corn. Scientists at Mississippi State have identified, developed, and released corn germplasm lines with resistance to aflatoxin contamination (Scott and Zummo 1990, 1992; Williams and Windham 2001). Germplasm was evaluated for resistance by inoculating developing corn ears with an *A. flavus* spore suspension 7–14 d after silk emergence and selecting for either reduced fungal infection or reduced aflatoxin accumulation.

This study was undertaken to investigate the association between southwestern corn borer (*Diatraea grandiosella* Dyar) damage and aflatoxin accumulation in corn hybrids artificially infested with southwestern corn borer and those not artificially infested and to compare performance of aflatoxin resistant lines in crosses with other lines.

Materials and Methods

Ten inbred lines of corn were selected as parents of a diallel cross. Two of the lines, Mp313E and Mp420, were released as sources of resistance to aflatoxin (Scott and Zummo 1990, 1992). A third line, Mp80:04, has also exhibited resistance to aflatoxin (unpublished data). The 10 inbred lines were crossed in all combinations, and the resulting 45 hybrids were evaluated for insect damage to the ears and aflatoxin contamination in 1999 and 2000 at Mississippi State, MS. On 22

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Table 1. Mean ratings of ear damage caused by feeding of Lepidoptera on corn hybrids grown at Mississippi State in 1999 and 2000

Hybrid	Ear damage ratings (mean ± SE)	
	1999	2000
Ab24E × Mp305	5.0 ± 1.2e-h	4.0 ± 1.4d-k
Ab24E × Mp313E	5.1 ± 1.0d-h	5.0 ± 1.7b-d
Ab24E × Mp339	5.4 ± 1.5c-f	4.1 ± 0.4c-j
AB24E × Mp420	5.0 ± 1.2e-h	3.0 ± 0.9k
Ab24E × Mp488	5.1 ± 1.4d-h	4.8 ± 1.7b-f
Ab24E × Mp80:04	4.4 ± 1.4i	3.6 ± 1.1g-k
Ab24E × SC212m	5.4 ± 1.2c-f	4.4 ± 1.3c-h
Ab24E × SC213	5.8 ± 1.8a-d	4.3 ± 1.2c-i
Ab24E × T165	6.1 ± 1.8ab	5.0 ± 1.3b-d
Mp305 × Mp313E	4.9 ± 0.8e-h	6.3 ± 1.5a
Mp305 × Mp339	5.9 ± 1.6a-c	4.5 ± 0.7c-i
Mp305 × Mp420	5.3 ± 1.0c-g	4.6 ± 0.9b-g
Mp305 × Mp488	4.6 ± 0.7g-i	3.9 ± 0.6e-k
Mp305 × Mp80:04	5.1 ± 1.1d-h	3.4 ± 0.9h-k
Mp305 × SC212m	4.6 ± 0.7g-i	3.1 ± 0.9jk
Mp305 × SC213	5.0 ± 0.9e-h	3.4 ± 1.2h-k
Mp305 × T165	5.1 ± 1.2d-h	3.9 ± 1.1e-k
Mp313E × Mp339	5.0 ± 1.2e-h	4.0 ± 1.6d-k
Mp313E × Mp420	5.4 ± 1.3c-f	4.1 ± 0.8c-g
Mp313E × Mp488	4.4 ± 0.9i	3.4 ± 1.2h-k
Mp313E × Mp80:04	5.1 ± 1.1d-h	4.5 ± 1.6c-g
Mp313E × SC212m	4.9 ± 1.4e-h	4.4 ± 1.4c-h
Mp313E × SC213	4.6 ± 1.1g-i	4.9 ± 1.6b-e
Mp313E × T165	4.6 ± 1.4g-i	3.3 ± 0.8i-k
Mp339 × Mp420	5.3 ± 1.9b-e	4.5 ± 1.3c-g
Mp339 × Mp488	4.9 ± 1.0e-h	5.1 ± 1.7b-c
Mp339 × Mp80:04	5.3 ± 1.0c-g	3.3 ± 0.7i-k
Mp339 × SC212m	5.1 ± 1.4d-h	3.6 ± 0.7g-k
Mp339 × SC213	5.4 ± 1.1c-f	4.0 ± 1.3d-k
Mp339 × T165	5.3 ± 1.0c-g	5.6 ± 0.9ab
Mp420 × Mp488	4.9 ± 1.0e-h	3.4 ± 1.3h-k
Mp420 × Mp80:04	4.5 ± 0.5h-i	3.0 ± 1.1k
Mp420 × SC212m	4.9 ± 1.2e-h	4.1 ± 1.0c-g
Mp420 × SC213	5.4 ± 1.5c-f	4.3 ± 1.3c-i
Mp420 × T165	5.8 ± 1.7a-d	4.0 ± 0.9d-k
Mp488 × Mp80:04	5.3 ± 1.0c-g	4.0 ± 1.6d-k
Mp488 × SC212m	4.8 ± 0.7f-i	3.4 ± 0.9h-k
Mp488 × SC213	5.1 ± 1.0d-h	3.4 ± 0.5c-h
Mp488 × T165	5.1 ± 1.4d-h	4.5 ± 1.5c-g
Mp80:04 × SC212m	4.9 ± 1.1e-h	3.9 ± 0.8e-k
Mp80:04 × SC213	5.3 ± 1.2c-g	4.6 ± 1.4b-g
Mp80:04 × T165	5.4 ± 1.2c-f	3.8 ± 0.9f-k
SC212m × SC213	6.4 ± 1.6a	5.1 ± 1.2bc
SC212m × T165	5.8 ± 1.6a-d	4.6 ± 1.2b-g
SC213 × T165	5.5 ± 1.5b-e	5.0 ± 1.2b-d
Mean	5.2	4.2

Ear damage caused by feeding of southwestern corn borer and other Lepidoptera was rated after harvest on a scale of 0 (no damage) to 9 (extensive damage). Values were obtained by averaging over southwestern corn borer infested and noninfested plots. Means in a column followed by the same letter do not differ at $P = 0.05$ (Fisher's protected LSD).

April 1999 and 27 April 2000, the hybrids were planted in a randomized complete block design with eight replications. The single row plots were 5-m long, spaced 0.97 m apart, and thinned to 20 plants.

Beginning when silks had emerged from 50% of the ears of the earliest maturing plots, all plants were inoculated with a spore suspension containing 9×10^7 *A. flavus* conidia per milliliter and a spreader sticker (Hi-Yield Chemical, Bonham, TX). Using a backpack sprayer (Solo, Newport News, VA), the suspension was applied to the silks and husks of the top ear of each plant weekly for 5 wk at the rate of ≈2 ml/plant/wk. *A. flavus* isolate NRRL 3357, which is known to produce aflatoxin in corn (Scott and Zummo 1988) was used as inoculum. The inoculum was prepared as described by Windham and Williams (1999).

Each year, 7 and 14 d after the initial application of *A. flavus,* plants in four replications were infested with 30 southwestern corn borer larvae from a laboratory colony maintained in the research unit (Davis 1997). On each day, 15 neonates each were placed in the leaf axil above the top ear, and 15 were placed in the axil of the top-ear leaf using a portable plastic dispensing device (Davis and Williams 1994).

The top ears were hand harvested ≈56 d after the initial *A. flavus* inoculation and dried at 38°C for 7 d. Ten ears from each plot were visually rated for feeding damage by southwestern corn borer and other Lepidoptera using the following rating scale: (1) no Lepidoptera damage to any ears, (2) only ear tip (<3 cm) damage to three or fewer ears, (3) ear tip damage to four to six ears and no damage below tips, (4) ear tip damage to most ears and light additional damage (two or three kernels at one or two sites destroyed) to three or fewer ears, (5) ear tip damage to most ears and light damage to kernels below tips of four to six ears, (6) ear tip damage and light damage and light damage below tips of most ears or moderate damage (four to six kernels destroyed at one to five sites) below tips of three or fewer ears, (7) ear tip damage and moderate to heavy damage (kernels destroyed at more than four sites or more than six kernels destroyed at fewer sites) to four to six ears, (8) ear tip damage and moderate to heavy damage below tips of seven or eight ears, and (9) ear tip damage and moderate to heavy damage below tips of nine to 10 ears.

Ears from each plot were shelled after insect damage was rated. The grain was mixed by pouring through a sample splitter twice. The grain was ground using a Romer mill (Union, MO). Aflatoxin contamination was determined in 50-g subsamples from each plot by the Vicam aflatest (Watertown, MA). This procedure detects aflatoxin at concentrations as low as 2 ng/g.

Plot means for ear damage and aflatoxin contamination were used in an analysis of variance (ANOVA). To equalize variances and because some values were zero, the data for aflatoxin contamination were transformed by adding one and taking the logarithm of each number [$\log(y + 1)$] before statistical analysis. Data on ear damage and aflatoxin were combined over years and treatments (with or without southwestern) for ANOVA using PROC GLM (SAS Institute 1996).

Because of significant interactions, additional analyses within years and treatments were completed. Means were compared using Fisher protected least significant difference (LSD) ($P = 0.05$) (Steel and Torrie 1980). Variation among hybrids was partitioned into general and specific combining ability using method 4, model I (Griffing 1956). Hybrids, years, and treatments were considered fixed effects.

Table 2. Mean ear damage ratings for each inbred line in crosses following artificial infestation with southwestern corn borer or without artificial infestation in 1999 and 2000 at Mississippi State

Inbred line	Noninfested plots		Infested plots	
	1999	2000	1999	2000
Ab24E	4.2 ± 0.4ab	3.8 ± 0.6ab	6.3 ± 0.7a-c	4.7 ± 0.8a
Mp305	4.2 ± 0.2ab	3.8 ± 1.0ab	5.9 ± 0.7c-e	4.3 ± 1.2ab
Mp313E	4.0 ± 0.3b	4.2 ± 0.8a	5.8 ± 0.4de	4.7 ± 1.1a
Mp339	4.3 ± 0.3a	4.0 ± 0.9a	6.3 ± 0.6a-c	4.5 ± 0.8ab
Mp420	4.1 ± 0.2ab	3.7 ± 0.8ab	6.2 ± 0.7a-d	4.1 ± 0.7b
Mp488	4.2 ± 0.3ab	3.7 ± 0.8ab	5.6 ± 0.5e	4.6 ± 0.9ab
Mp80:04	4.2 ± 0.4ab	3.3 ± 0.8b	5.9 ± 0.4c-e	4.3 ± 0.8ab
SC212m	4.3 ± 0.4a	3.7 ± 0.5ab	6.1 ± 0.9b-d	4.4 ± 0.8ab
SC213	4.3 ± 0.4a	4.1 ± 0.5a	6.5 ± 0.7ab	4.8 ± 0.9a
T165	4.2 ± 0.4ab	4.1 ± 0.7a	6.6 ± 0.7a	4.7 ± 1.1a

Ear damage caused by feeding of southwestern corn borer and other Lepidoptera was rated after harvest on a scale of 1 (no damage) to 9 (extensive damage). Means in a column followed by the same letter do not differ at $P = 0.05$ (Fisher's protected LSD).

Results and Discussion

Ear damage caused by feeding of southwestern corn borer and other Lepidoptera was significantly greater in 1999 than in 2000 ($F = 40.62$; df = 1, 12; $P < 0.01$); therefore, ear damage ratings were further analyzed within years. In 1999, the mean damage rating for hybrids artificially infested with southwestern cornborer was 6.1 ± 0.4. The mean rating for hybrids grown in noninfested plots was 4.2 ± 0.3; this indicates a significant level of natural infestation by southwestern corn borer or other Lepidoptera. The average rating for each hybrid is given in Table 1. Differences among hybrids were highly significant ($F = 3.1$; df = 44, 264; $P < 0.01$). Ab24E x Mp80:04 and Mp313E x Mp488 sustained the least damage (4.4); SC212M x SC213 sustained the heaviest damage (6.4) and Ab24E x T165, the second heaviest (6.1). In 2000, the mean ear damage ratings for artificially infested and noninfested plots were 4.5 ± 0.6 and 3.8 ± 0.5, respectively. Ab24E x Mp420 and Mp420 × Mp80:04 sustained the least damage (3.0) and Mp305 × Mp313E, the heaviest damage (6.3) in 2000 (Table 1).

The mean ear damage ratings for each inbred line in crosses with the other lines are given in Table 2. Differences among the inbred lines were generally small. The diallel analysis indicated that both general combining ability ($F = 5.66$; df = 9, 520; $P < 0.01$) and specific combining ability ($F = 4.21$; df = 35, 520; $P < 0.01$) were highly significant sources of variation. Estimates of general combining ability effects indicated that Mp80:04, Mp420, and Mp488 contributed to reduced ear damage and SC213 and T165 contributed to greater damage (Table 3).

Table 3. Estimates of general combining ability effects for ear damage in 1999 and 2000 and aflatoxin accumulation in 2000

Inbred line	Ear damage	Aflatoxin [log(ng/g + 1)]
Ab24E	0.09	0.57**
Mp488	−0.17*	0.34**
SC213	0.26**	0.34**
SC212m	−0.01	0.30**
Mp305	−0.11	0.06
Mp339	0.13	0.01
Mp420	−0.16*	−0.18
T165	0.28**	−0.21
Mp313E	0.00	−0.48**
Mp80:04	−0.28**	−0.74**

*, **, Indicate significant difference from 0 at $P = 0.05$ and 0.01, respectively.

Although insect damage was higher in 1999 than in 2000, aflatoxin levels were much lower in 1999. In the experiment that was not infested with southwestern corn borer, no aflatoxin was detected in 27 of 45 hybrids. The overall mean aflatoxin level was only 2 ng/g. When infested with southwestern corn borer, aflatoxin levels were significantly higher ($F = 89.21$; df = 1, 260; $P < 0.01$) than in the nonsouthwestern corn borer infested plots. The overall mean aflatoxin level for southwestern corn borer infested hybrids was 5 ng/g. Because of the unusually low aflatoxin levels in 1999, these data were not analyzed further.

Aflatoxin levels were significantly higher in 2000 ($F = 29.06$; df = 1, 2; $P < 0.01$) than in 1999. The overall mean aflatoxin level for hybrids inoculated with *A. flavus*, but not infested with southwestern corn borer was 164 ng/g. Aflatoxin levels for these hybrids ranged from 24 ng/g for Mp313E x T165–710 ng/g for SC212M x SC213 (Table 4). Aflatoxin levels for the three classes of hybrids were 309 ± 211 for susceptible x susceptible (SxS), 151 ± 122 for susceptible x resistant (SxR), and 113 ± 69 for resistant x resistant (RxR). When infested with southwestern corn borer, aflatoxin levels were significantly higher ($F = 18.91$; df = 1, 262; $P < 0.01$). The overall mean was 254 ng/g. Ab24E x SC213 had the highest level of aflatoxin accumulation (1,251 ng/g) and Mp339 × Mp80:04, the lowest (30 ng/g). Aflatoxin levels for the three classes of hybrids were 435 ± 241 ng/g (SxS), 214 ± 105 ng/g (SxR), and 138 ± 113 ng/g (RxR).

Aflatoxin levels for the SxR crosses were ≈50% of those for the SxS crosses both with and without southwestern corn borer infestation, and aflatoxin levels for the RxR crosses were ≈35% of those for the SxS crosses. These results indicate that one resistant parent can substantially reduce aflatoxin levels. In crosses with other inbred lines, the resistant lines Mp313E and Mp80:04 exhibited the lowest levels of aflatoxin accumulation with or without southwestern corn borer

Table 4. Geometric means for aflatoxin accumulation in hybrids inoculated with *A. flavus* and artificially infested with southwestern corn borer or not infested in 2000

Hybrid	Classification[a]	Aflatoxin (ng/g)	
		A. flavus[b]	*A. flavus* + southwestern corn borer[c]
Ab24E × Mp305	S × S	280 a-j	427 a-g
Ab24E × Mp313E	S × R	395 a-f	190 d-j
Ab24E × Mp339	S × S	263 a-j	357 b-h
Ab24E × Mp420	S × R	102 e-1	294 b-h
Ab24E × Mp488	S × S	878 a	596 a-c
Ab24E × Mp80:04	S × R	110 d-k	441 a-g
Ab24E × SC212m	S × S	305 a-h	508 a-e
Ab24E × SC213	S × S	436 a-c	125 1a
Ab24E × T165	S × S	199 b-j	344 b-h
Mp305 × Mp313E	S × R	139 d-k	300 b-h
Mp305 × Mp339	S × S	298 a-i	228 c-i
Mp305 × Mp420	S × R	73 h-l	303 b-h
Mp305 × Mp488	S × S	339 a-g	573 a-d
Mp305 × Mp80:04	S × R	136 d-k	189 d-j
Mp305 × SC212m	S × S	167 b-j	196 c-j
Mp305 × SC213	S × S	138 d-k	206 c-i
Mp305 × T165	S × S	123 d-k	226 c-i
Mp313E × Mp339	R × S	108 e-k	53 1m
Mp313E × Mp420	R × R	192 b-j	268 b-h
Mp313E × Mp488	R × S	280 a-j	187 d-j
Mp313E × Mp80:04	R × R	79 g-l	65 j-m
Mp313E × MpSC212m	R × S	113 d-k	274 b-h
Mp313E × SC213	R × S	98 f-l	164 f-k
Mp313E × T165	R × S	24 l	57 k-m
Mp339 × Mp420	S × R	168 b-j	300 b-h
Mp339 × Mp488	S × S	382 a-f	588 a-c
Mp339 × Mp80:04	S × R	34 kl	30 m
Mp339 × SC212m	S × S	197 b-j	387 b-g
Mp339 × SC213	S × S	233 a-j	290 b-h
Mp339 × T165	S × S	682 a-c	337 b-h
Mp420 × Mp488	R × S	79 g-l	249 c-h
Mp420 × Mp80:04	R × R	67 j-l	81 i-m
Mp420 × SC212m	R × S	472 a-d	184 c-j
Mp420 × SC213	R × S	354 a-f	153 g-l
Mp420 × T165	R × S	161 c-j	154 g-l
Mp488 × Mp80:04	S × R	92 f-l	332 b-h
Mp488 × SC212m	S × S	104 e-l	338 b-h
Mp488 × SC213	S × S	230 a-j	427 a-g
Mp488 × T165	S × S	67 j-l	492 a-f
Mp80:04 × SC212m	R × S	69 i-l	342 b-h
Mp80:04 × SC213	R × S	121 d-k	184 e-j
Mp80:04 × T165	R × S	34 k-l	122 h-l
SC212m × SC213	S × S	710 ab	801 ab
SC212m × T165	S × S	179 b-j	516 a-e
SC213 × T165	S × S	308 a-h	238 c-i
Mean		164	254

Means in a column followed by the same letter do not differ at $P = 0.05$. Tests of significance were performed on transformed [log(y + 1)] means using Fisher's protected LSD before converting values to the original scale.

[a] R indicates a line selected for resistance to aflatoxin contamination; S indicates a susceptible line.

[b] An *A. flavus* spore suspension was sprayed on the silks and husks of the top ear of each plant weekly for 5 wk beginning when the earliest plots reached midsilk.

[c] An *A. flavus* spore suspension was applied weekly for 5 wk and top ears were infested with 30 southwestern corn borer larvae following the second and third applications of the spore suspension.

Table 5. Mean aflatoxin accumulation for each inbred line in crosses with other inbreds following inoculation with *A. flavus* and with or without southwestern corn borer infestation in 2000

Hybrid	*A. flavus*	*A. flavus* + southwestern corn borer
Ab24E	267 a	429 a
Mp305	168 a-c	277 b-d
Mp313 E	123 cd	141 f
Mp339	201 ab	214 de
Mp420	149 bc	207 d-f
Mp80:04	75 d	149 ef
SC212m	200 a-c	359 ab
SC213	244 ab	315 a-c
T165	127 c	229 cd

Means in a column followed by the same letter do not differ at $P = 0.05$. Tests of significance were performed on transformed [log(y + 1)] means using Fisher's protected LSD before converting values to the original scale.

infestation (Table 5). Crosses with Ab24E as one parent had the highest levels of contamination in both situations.

Both general combining ability (GCA) and specific combining ability (SCA) were significant sources of variation in the inheritance of resistance to aflatoxin accumulation (GCA: $F = 12.34$; df = 9, 262; $P < 0.01$ and SCA: $F = 2.08$; df = 35, 262; $P < 0.01$). Estimates of GCA effects indicate that Mp313E and Mp80:04 contributed significantly to aflatoxin-resistant hybrids (Table 3).

The relationships among corn genotypes, fungi, and insects are complex. The results of the current investigation are consistent with the results of earlier investigations. In a 2-yr study conducted at five locations, Barry et al. (1992) found that although insect damage and aflatoxin contamination varied among environments, the corn genotypes that sustained less insect damage also exhibited lower levels of aflatoxin contamination. The insect damage was attributed primarily to corn earworm, *Helicoverpa zea* (Boddie). Among corn hybrids genetically engineered for resistance to European corn borer, *Ostrinia nubilalis* (Hübner), Munkvold et al. (1997) reported that Fusarium ear rot incidence was positively correlated with European corn borer kernel damage. Windham et al. (1999) found that southwestern corn borer infestation and *A. flavus* inoculation techniques, placement of the insect and the fungus, and the timing of the infestation/inoculation were all important in establishing the role that southwestern corn borer plays in aflatoxin contamination.

The results of this investigation indicate that southwestern corn borer feeding can result in increased aflatoxin contamination, but environmental conditions must be conducive to aflatoxin contamination to see this effect. Insect damage was greater in 1999 than in 2000, but there was very little aflatoxin in 1999. In 2000, when environmental conditions were apparently more favorable for aflatoxin production, the increased damage from southwestern corn borer sustained by the hybrids, resulted in higher levels of aflatoxin contamination. Although the estimated general combining ability effects (Table 3) indicate that Mp80:04 contributed significantly to reduced ear damage from insect feeding and reduced aflatoxin levels, Mp313E contributed to reduced aflatoxin contamination, but not reduced ear damage. Crosses with Mp488 exhibited significantly less ear damage, but significantly greater aflatoxin contamination. None of the lines included in this study exhibited high levels of resistance to southwestern corn borer. The aflatoxin-resistant lines Mp313E and Mp80:04 should be useful in developing resistant hybrids. Combining the resistance in these lines with genetic resistance to southwestern corn borer and other Lepidoptera could result in an even greater reduction in losses to aflatoxin.

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